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研究課題名（和文）Where is stressed processed in brain during sleep? -- A Neuroimaging study into stress-related abnormalities in brain activity during sleep

研究課題名（英文）Where is stressed processed in brain during sleep? -- A Neuroimaging study into stress-related abnormalities in brain activity during sleep

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研究成果の概要（和文）：この研究では、就寝時のストレスと最初の睡眠サイクル中の前頭前野（PFC）の血行動態との関連を調査しました。唾液コルチゾールとsIgAを含むストレスバイオマーカーが測定されました。主観的なストレスレベルは、唾液サンプルの収集直後に1-10のリッカート尺度で評価されました。前頭前野（PFC）の血行動態は、ウェアラブル機能的近赤外分光法（fNIRS）デバイスを使って測定されました。ストレスは、中央DLPFC、尾側DLPFC、および左RLPFCの血行動態と相関することがわかりました。ストレスとこれらのPFCサブ領域の関係は、採用されているストレス指標によって異なります。

研究成果の学術的意義や社会的意義
This study expands the scope of stress studies by examining how sleep modulates brain's response to stress during sleep. The findings will inform the development of new interventions for stress-related sleep problems.

研究成果の概要（英文）：This study explored the associations between bedtime stress and the hemodynamics in the prefrontal cortex (PFC) during the first sleep cycle. Stress biomarkers including salivary cortisol and sIgA were measured. Perceived stress level was rated on a 1-10 Likert scale right after the collection of the salivary samples. The hemodynamics of the pre-frontal cortex (PFC) was measured using a wearable functional near-infrared spectroscopy (fNIRS) device. Stress was found to correlate to the hemodynamics in the mid-DLPFC, the caudal-DLPFC, and the left RLPFC. The relationships between stress and these PFC subregions depends on the stress indicator adopted. Our finding provides supplementary support to the role of the PFC in processing stress.

研究分野：応用健康科学

キーワード：fNIRS　stress　sleep　brain imaging　personal informatics　wearable computing　ubiquitous computing

１．研究開始当初の背景

Stress is a worldwide epidemic with major health consequences. Stress is also known to cause sleep problems, suggesting abnormalities in brain activity during sleep in response to stress. Understanding the neurophysiological mechanism that underlies the relation between stress and sleep may give hint to the development of brain activity markers of stress, which can be readily measured and monitored using wearable brain-computer interface technologies. Findings from previous neuroimaging studies conducted in wake time has shed light on the role of the prefrontal cortex (PFC) in responding to acute stress. Nevertheless, no study has looked at stress-related abnormality in brain during sleep. A main reason for this knowledge gap is that conducting brain scanning during sleep is difficult using traditional brain imaging techniques (e.g., fMRI and PET), because participants could hardly fall asleep in noisy and vibrating scanners. Recent advances in alternative neuroimaging techniques such as functional near-infrared spectroscopy (fNIRS) and wearable technologies have led to a promising solution to overcome the limitations of traditional techniques.

２．研究の目的

In this study, we aimed to explore the association between bedtime stress and the hemodynamic responses in the PFC during the first cycle of nocturnal sleep We quantify stress level using both objective and subjective indicators. This study serves to validate the feasibility of using wearable fNIRS to explore brain activity in daily life settings, as well as generating preliminary suggestion to the development of new stress response markers in cortical hemodynamics during sleep. We especially focus on answering the following two research questions: (1) what feature variables derived from the hemodynamic responses in the PFC are significantly associated to the level of stress biomarkers and to perceived stress, respectively? (2) which subregions in the PFC are significantly associated to the level of stress biomarkers and to perceived stress, respectively?

３．研究の方法

A. Measuring Stress and Brain Activity

Salivary cortisol and sIgA concentration were measured using the SOMA Dual Analyte LFD test kits. These kits can be used for real-time measurement of cortisol and sIgA conveniently in a naturalistic setting. Saliva samples were collected using oral fluid collector (OFC) swabs and were incubated for 15 minutes in OFC buffers before being read. The participant was instructed not to eat, drink, or brush teeth 30 min prior to providing a saliva sample. The limit of detection (LOD) is 0.17 nM for cortisol and 18.10 μg/mL for sIgA. The limit of quantification (LOQ) is 0.58 nM for cortisol and 24.30 μg/mL for sIgA. The calibration ranges are 1.25-40 nM for cortisol and 25-800 μg/mL for sIgA. In addition to the measurement of stress biomarkers, we also collected data on perceived stress using a 1-10 Likert scale, with larger value indicating higher level of perceived stress. This scale was implemented using a mobile app called Health Log.

Brain activity as characterized by cortical hemodynamics was measured using a wearable functional near-infrared spectroscopy (fNIRS) (Brite 24; Artinis Medical Systems Co., The Netherlands), which measures the concentration changes in oxyhemoglobin (ΔO2Hb), deoxyhemoglobin (ΔHHb),

and total hemoglobin (ΔHbt) in cortical brain areas. The fNIRS used in this study has 10 transmitters (Tx) and 8 receivers (Rx). The Txs take turns to emit infrared light at two wavelengths: 760 nm and 850 nm. Part of the infrared light gets scattered or absorbed as it travels through the human tissue of the head (i.e., sculp, skull, and cortex). The rest returns to the surface of the head and is captured by the Rxs. The Txs and Rxs were configured into 27 channels. The optodes were placed between the FpZ-F3-Cz-F4-FpZ region in the prefrontal cortex according to the international 10-20 EEG system with an interoptode distance of 3 cm (aka. penetration depth = 1.5 cm). They were fixed on a soft neoprene head cap, which ensures the alignment of optode placement across different measurements. A Fitbit Sense was used to record the start of sleep. Previous studies have demonstrated the validity of Fitbit in detecting sleep onset.

B. Data Collection Protocol

Grounded on the N-of-1 trial method, a longitudinal data collection experiment was conducted with one healthy participant (male, 36 years). The data collection experiment lasted 15 days. This study has been approved by the Ethics Committee of the Kyoto University of Advanced Science and written informed consent was obtained from the participant before the experiment started. On the nights that the data collection experiment was conducted, we took saliva samples from the participant before he went to bed. We ensured that the samples were always collected during a fixed time period between 22:00-23:00, as cortisol and sIgA level follow a 24-hour rhythm. It is hence highly recommended to measure these hormones at a constant time of the day to cancel out the confounding effect of the circadian rhythm. Right after taking the saliva sample, the participant was asked to rate how stressful he felt on the Health Log app. When the participant was ready to go to bed, we set up the Brite 24 on his head, started the device and put the Fitbit Sense on his non-dominant wrist. The participant was asked to firstly sit quietly on bed while staying awake for 2 minutes. The purpose of the awake rest phase is to reset the brain to a common baseline, as the fNIRS device measures the concentration changes—not the absolute concentration—of the oxyhemoglobin and deoxyhemoglobin. After the awake rest phase, the participant then got in bed and turned off the light. The Brite 24 was left on until it ran out of battery. The participant was instructed to take off and stop the Brite 24 (simply by pressing the main button) when he needed to go to the restroom early morning or when he woke up, whenever which happened first. It is worth noting that the participant shaved his hair to eliminate the interference of dark hair on the signal quality of the Brite 24.

C. Data Analysis Protocol

The measured salivary cortisol and sIgA data were manually input into a csv file. The perceived stress levels were exported from the Health Log app into a csv file with a premium account. The two files were then merged through matching date stamps. The fNIRS data were first exported from the device in the form of raw optical density (OD) data. Bad channels were removed using the scalp coupling index method with 0.75 as the cut-off threshold. The OD data were then transformed to ΔO2Hb and ΔHHb using the modified Beer-Lambert law (MBLL). Breath and heartbeat noise were removed using a bandpass filter with cut-off frequencies 0.02-0.18 Hz. Time and frequency domain features were then derived from each channel. The fNIRS data processing pipeline was implemented in Python 3.8.8.

TABLE I. VARIABLES USED IN CORRELATION ANALYSIS

Category	Metric (Denotation)	Data Type	Device & Instrument
Stress	Salivary cortisol (*SC*)	Continous	SOMA Dual Analyte LFD
	Salivary sIgA (*SA*)		
	Perceived stress (*PS*)	Ordinal	1-10 Likert scale
Brain activity as indicated by ΔO2Hb and ΔHHb signals	Mean (*mean_O2*/*mean_H*)	Continous	Brite 24
	Median (*md_O2*/*md_H*)		
	Standard deviation (*sd_O2*/*sd_H*)		
	Skewness (*sk_O2*/*sk_H*)		
	Kurtosis (*kt_O2*/*kt_H*)		
	Total power (*tp_O2*/*tp_H*)		
	Maximum frequency (*mf_O2*/*mf_H*)		
	Peak ratio (*pr_O2*/*pr_H*)		

The stress data and the features derived from the fNIRS data were all merged into an csv file by matching the date stamps. Table II provides a list of all the variables that were used in the subsequent correlation analysis. Pearson' correlation coefficients were calculated pair-wisely on continuous variables, and Spearman's correlation coefficients were calculated on variables at ordinal levels. The significance of the correlation coefficients (significance level $\alpha = 0.05$) was tested to decide whether the values of the coefficients were significantly different from zero. Correlation coefficient matrix was calculated using the Pandas python library, which was then used to create a heatmap using the Seaborn python library. The statistical test on the correlations was performed using the SciPy library.

４．研究成果

In total 15 days of data were collected. Table II presents the statistically significant correlations between stress indicators and the features derived from the cortical hemodynamics. A rough visualization of the suppressed and aroused subregions is provided in Figure 1.

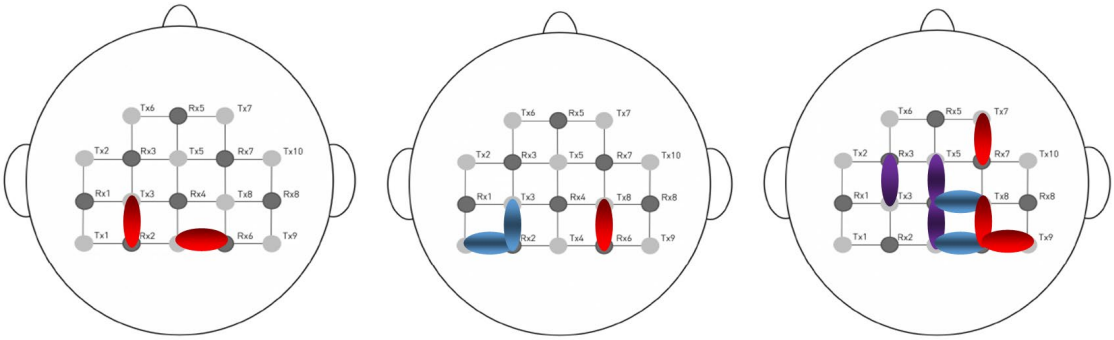
Correlation analysis results suggested two main findings. First, the standard deviation, skewness, and kurtosis of the ΔO2Hb and the ΔHHb averaged across all channels were significantly correlated, suggesting a possible phase synchronization between the two types of hemoglobin in the PFC during the first sleep cycle. Such linear correlation between the ΔO2Hb and the ΔHHb has been previously observed when participants underwent visual stimulations in wake time. Second, the relation between stress and the hemodynamics in the PFC depends on the stress indicator adopted. Higher salivary cortisol level was associated to increased concentration change of the oxyhemoglobin in both the left and the right DLPFC, suggesting stress-associated abnormal arousal in these subregions. Lower sIgA was associated to increased mean concentration change of oxyhemoglobin in the whole measured region and the median concentration change of oxyhemoglobin in the left caudal DLPFC, suggesting stress-associated arousal in these regions. On the other hand, lower sIgA was associated to lower peak ratio of the concentration change of deoxyhemoglobin and thus suggests possible suppression of the right caudal DLPFC.

In comparison to stress biomarkers, perceived stress was associated to more subregions in the PFC during the first sleep cycle. Higher perceived stress was associated to arousal in the left RLPFC,

the left mid-DLPFC, and the left caudal-DLPFC, as well as suppression in the left-caudal-DLPFC. Higher perceived stress was correlated to increased dynamics of the deoxyhemoglobin but suppressed dynamics of the oxyhemoglobin in the mid-DLPFC, as well as increased dynamics of oxyhemoglobin in the caudal-DLPFC.

TABLE II. STATISTICALLY SIGNIFICANT CORRELATIONS BETWEEN BEDTIME STRESS AND HEMODYNAMIC FEATURES

Metric 1	***Metric 2***	***r***	***p***	***Metric 1***	***Metric 2***	***r***	***p***
SC	*md_O2* (Rx2-Tx3)	0.631	0.015	*PS*	*pr_O2* (Rx4-Tx4)	-0.636	0.011
	pr_O2 (Rx6-Tx4)	0.561	0.037		*mean*_H (Rx4-Tx5)	0.610	0.016
SA	*mean_O2*	-0.578	0.024		*sd_H* (Rx4-Tx5)	-0.597	0.019
	md_O2 (Rx2-Tx1)	-0.522	0.046		*tp_H* (Rx4-Tx5)	-0.597	0.019
	md_O2 (Rx2-Tx3)	-0.549	0.034		*sd_O2* (Rx4-Tx8)	-0.681	0.005
	pr_H (Rx6-Tx8)	0.568	0.027		*tp_O2* (Rx4-Tx8)	-0.681	0.005
PS	*sd_O2* (Rx3-Tx3)	-0.574	0.025		*mf_O2* (Rx4-Tx8)	-0.651	0.009
	sd_H (Rx3-Tx3)	0.521	0.046		*mf_H* (Rx6-Tx4)	-0.538	0.038
	tp_O2 (Rx3-Tx3)	-0.574	0.025		*mean_H* (Rx6-Tx8)	0.630	0.021
	tp_H (Rx3-Tx3)	0.564	0.029		*md_H* (Rx6-Tx8)	0.647	0.009
	sd_O2 (Rx4-Tx4)	-0.548	0.035		*sd_O2* (Rx6-Tx9)	0.67	0.006
	sk_O2 (Rx4-Tx4)	0.672	0.006		*mf_O2* (Rx6-Tx9)	0.572	0.026
	tp_O2 (Rx4-Tx4)	-0.548	0.035		*mean_H* (Rx6-Tx9)	0.652	0.041
	mf_O2 (Rx4-Tx4)	-0.655	0.008		*mean_O2* (Rx7-Tx7)	0.717	0.046



(a) (b) (c)

Figure 1. Visualization of channels in which the measured cortical hemodynamics was positively (red) and negatively (blue) associated to different stress indicators. Purple indicates that both arousal and suppression was observed. (a) salivary cortisol, (b) sIgA, (c) perceived stress.

５．主な発表論文等

〔雑誌論文〕　計9件（うち査読付論文　9件／うち国際共著　3件／うちオープンアクセス　3件）

１．著者名 Liang Z	４．巻 0
２．論文標題 A n N of 1 Investigation into Stress Related Hemodynamic Response s in the Prefrontal Cortex During the First Sleep Cycle	５．発行年 2021年
３．雑誌名 Proceedings of the IEEE International Conference on Systems, Man and Cybernetics	６．最初と最後の頁 1-6
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Liang Zilu、Chapa-Martell Mario Alberto	４．巻 3
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〔図書〕　計0件

〔産業財産権〕

〔その他〕

Personal Website
https://www.zilu-liang.net/research-projects
ResearchGate Profile
https://www.researchgate.net/profile/Zilu_Liang
Google Scholar Profile
https://scholar.google.co.jp/citations?user=UlMDCkQAAAAJ&hl=ja
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https://dblp.org/pers/l/Liang:Zilu.html

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関